Controlling mass mortality events with probiotics during the blue mussels (*Mytilus edulis*) larvae rearing process: what role is played by the larval microbiota?

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1. Introduction

- Blue Mussels (*Mytilus edulis*) production in hatcheries (figure 1) is limited by the occurence of mass mortality events which are generally related to the presence of bacterial pathogens in the rearing system.
- Culture conditions in the rearing system can lead to the development of opportunistic pathogens, such as *Vibrio splendidus,* at a high density.
- Despite its effectiveness, the use of antibiotics poses many problems in aquaculture (e.g. occurrence and transmission of antibiotics resistance in the food web, long-term inefficiency, etc…) and is highly regulated internationally.
- The use of probiotics such as marennine, a blue pigment produced by *Haslea ostrearia* (figure 2), could be a promising alternative to antibiotics in bivalve hatcheries.[1]

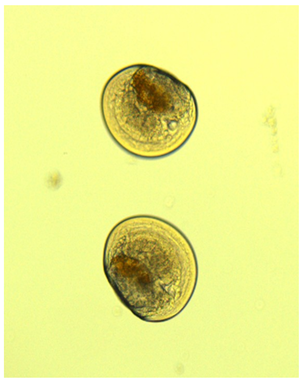


Figure 1. Blue mussel D-larvae (Latour ©)

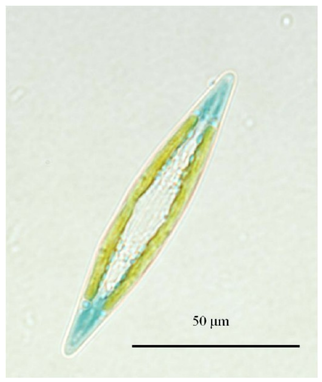
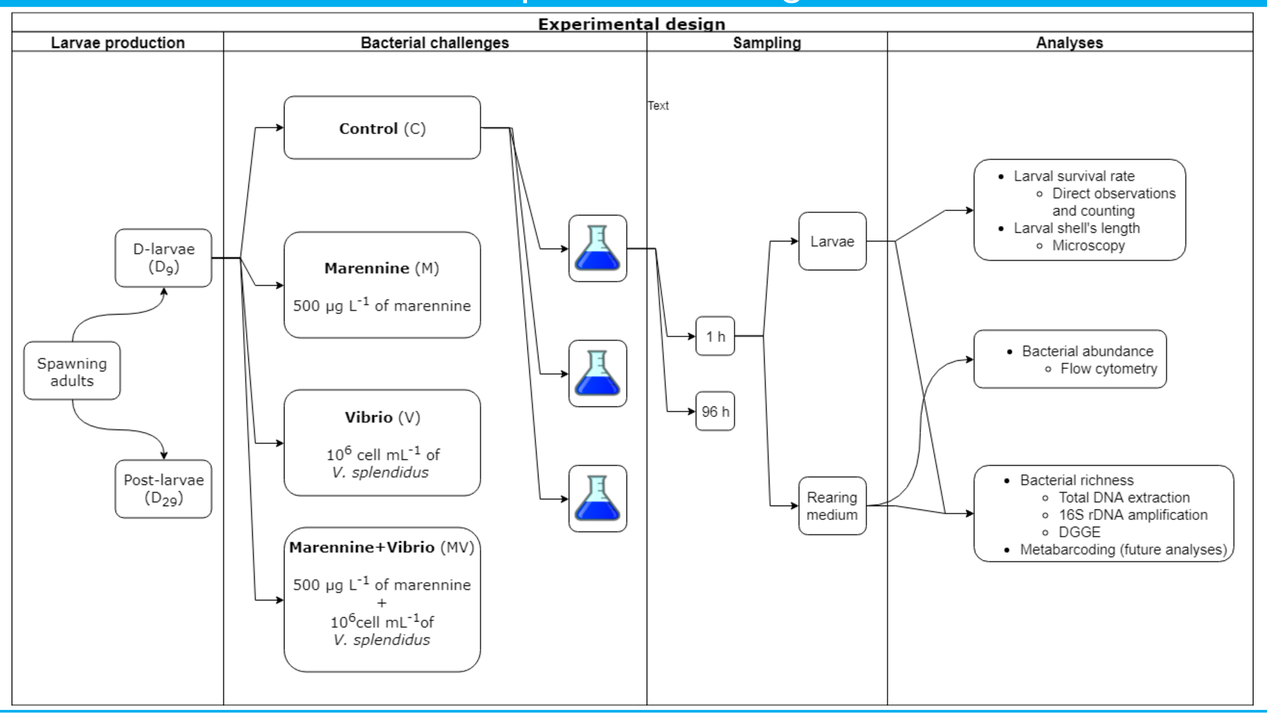


Figure 2. *Haslea ostrearia*[2]

2. Main objective of the study

Highlighting the protective effect of a new natural probiotic, marennine, on *Mytilus edulis* larvae during bacterial challenges in relation to a potential modification of the microbiota of the marennine-treated larvae

3. Experimental design



4.1. Larval survival rate

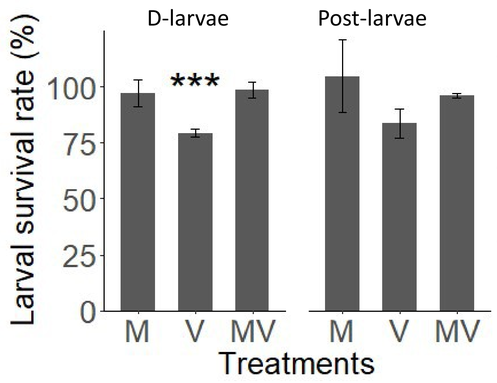


Figure 3. Larval survival rate (%) for D-larvae and post-larvae after 96 h of exposition during the bacterial challenges. Significant differences are shown with *** when p<0.05).

- The presence of the pathogen *V. splendidus* decreased the larval survival rate after 96h of exposition for the unchallenged D-larvae but not for the post-larvae
- Marennine desmonstrated a protective effect on the challenged D-larvae
- A preliminary experiment has demonstrated that marennine have no direct antibacterial effect on *V. splendidus* (data not shown)

Marennine-treated D-larvae were protected against *V. splendidus* during the experiments even though marennine did not previously show a direct antibacterial effect

4.2. Bacterial abundance

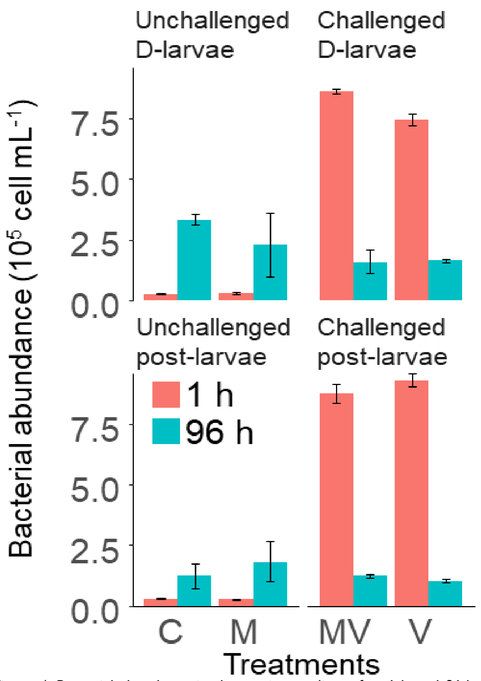


Figure 4. Bacterial abundance in the rearing medium after 1 h and 96 h of exposition of a) the unchallenged D-larvae, b) the challenged D-larvae against, c) the unchallenged post-larvae and d) the challenged post-larvae. Standard deviation is shown with error bars.

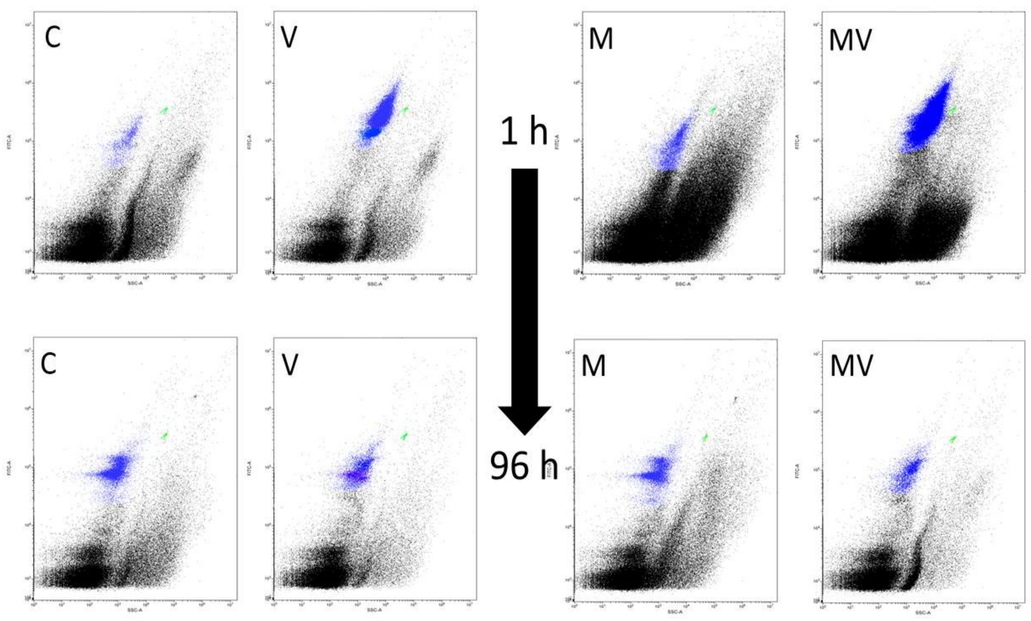


Figure 5. Cytograms obtained from the flow cytometry analyses for each treatments after 1 h and 96 h of exposition. The events in blue are considered as bacterial cells and the events in green are from the internal standard used.

- The presence of marennine did not affect the abundance of bacterial cells
- The addition of *V. splendidus* cells is tracable with the cytograms after 1 h but not after 96 h

Marennine did not demonstrate a direct antibacterial effect when used during the bacterial challenges of both larval stages against *V. splendidus* suggesting its effect is "in the larvae"

4.3. Bacterial richness

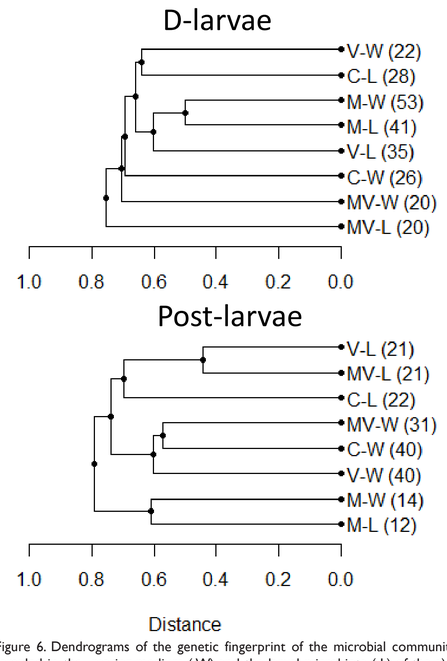


Figure 6. Dendrograms of the genetic fingerprint of the microbial communities sampled in the rearing medium (-W) and the larval microbiota (-L) of the a) D-larvae and the b) post-larvae after 96 h of exposition to the 4 differents conditions. The cluster analyses were based on the Jaccard coefficient similarity and the dendrograms were constructed with the UPGMA algorithm. Numbers between parentheses are the numbers of OTUs.

- The microbiota's genetic fingerprint from challenged D-larvae exposed to marennine was highly dissimilar (~80%)
- The presence of marennine generally lead to a diminution of the number of OTUs detected

The presence of marennine modified the genetic fingerprint of the challenged D-larvae's microbiota suggesting that the protective effect of marennine might come from a modification of the larval microbiota

5. Conclusion

The results demonstrated that the presence of marennine in the rearing medium of the challenged D-larvae had a protective effect which is associated with a modification in the larval microbiota' genetic fingerprint. Metabarcoding analyses will enable us to investigate the latter larval microbiota modification.

References
1. Turcotte F, Mouget J-L, Genard B, Lemarchand K, Deschênes J-S, Tremblay R. 2016. Aquatic Living Resources 29:401.
2. Gastineau R, Turcotte F, Pouvreau JB, Morancais M, Fleurence J, Windarto E, Prasetiya FS, Arsad S, Jaouen P, Babin M, Coiffard L, Couteau C, Bardeau JF, Jacquette B, Leignel V, Hardivillier Y, Marcotte I, Bourgougnon N, Tremblay R, Deschenes JS, Badawy H, Pasetto P, Davidovich N, Hansen G, Dittmer J, Mouget JL. 2014. Mar Drugs 12:3161-3189.